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Detection of lamivudine and adefovir resistance mutations among Filipino patients with chronic hepatitis B using line probe assay

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Background: Hepatitis B virus (HBV) mutations conveying resistance to antivirals are a major health concern worldwide in the treatment of chronic hepatitis B infection. The availability of a sensitive assay that can detect emerging HBV antiviral resistance mutations is important for individualized monitoring of response to standard of care treatment. The risk of developing lamivudine (LAM) resistance is 14% to 70% after 1 to 5 years of treatment, which is sufficiently high to justify HBV antiviral resistance mutation testing. On the other hand, resistance to adefovir (ADV) is slow to emerge, ranging from 3% to 29% following 1 to 5 years of treatment. Mutation analysis helps in decision-making for clinical management of chronic HBV infection and molecular epidemiological studies as well. This study seeks to determine the presence of LAM and ADV resistance mutations among Filipino patients clinically diagnosed with chronic hepatitis B from July 2008 to December 2011.

Methods: A total of fourteen samples were examined for the presence of antiviral resistance mutations using line probe assay, INNO-LiPA HBV DR v2. There were 9 (64%) males and 5 (36%) females with ages ranging from 17 to 69 years old.

Results: Among the fourteen samples, 5 (36%) had wild-type, 4 (28%) had HBV mutant strain DNA not detected, and 5 (36%) had HBV DNA mutations. Out of the 5 HBV DNA mutant strains, one or 20% had one-drug resistance mutation detected (rtA181T for ADV). Three or 60% had 3-drug resistance mutations pattern detected. The first patient had rtG173L for LAM compensatory mutation, rtL180M for LAM, and rtM204V for LAM. The second patient had rtL80I for LAM compensatory mutation, rtL180M for LAM, and rtM204I for LAM, telbivudine (TBV). The third patient had L180L/A181T upcoming resistance for ADV, M204I upcoming resistance for LAM, emtricitabine, TBV, and N236T upcoming resistance for ADV. The remaining one or 20% had 4 drug-resistance mutations detected (rtA181T for ADV, rtL180M for LAM, rtM204V for LAM, and rtM204I for LAM, TBV).

Conclusion: The most frequently encountered LAM-resistant mutant was rtM204V/I. Overall, the line probe assay provides a useful tool for the detection of lamivudine and adefovir resistance mutations among HBV-infected Filipino patients.

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Bioinformatics prediction of siRNAs as potential antiviral agents against dengue viruses

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Background: Dengue viruses (DENV 1 -4) represent the major emerging arthropod-borne viral infection in the world. Currently, there is neither a specific treatment nor an approved or available vaccine for these viral infections. The main problem for developing dengue vaccines is their inability to provide simultaneous protection against the four distinct viral serotypes. For this reason, there is a need to develop antiviral drugs for treating this disease. We propose the use of RNA interference (siRNA), targeting highly conserved regions among the four DENV serotypes to silence the viral genomes.

Methods: To identify highly conserved regions in the genome of the four DENV serotypes, a multiple alignment with Clustal X program was carried out, using all DENV genome sequences available at NCBI data base, previously depurated by discarding identical genome sequences with the virtual hybridization program. For the siRNA design, four servers were used: Invitrogen RNAi Designer, Dharmacon siRNA Design Center, Qiagen Design Tool and Ambion, Inc. Prediction of the genome's secondary structure for each serotype was obtained with the RNA structure program.

Results: A total of 2,893 complete DENV genomes were downloaded and after depuration 220 genomes were left. The highly conserved zone among the four serotypes was found to be located in the region corresponding to the NS4b and NS5 proteins. A consensus sequence from these regions was used to siRNA design. Three siRNAs that met most of criteria proposed for RNA silencing were chosen. One targets the genome region that codifies for NS4b protein and the other two, the region for NS5protein. To assure that the siRNAs were specific for the relevant genome region; we performed a BLAST analysis against *homo sapiens* database. Prediction of the genome's secondary structure was used to demonstrate that the predicted siRNAs were complementary to the target site in the viral genome.

Conclusion: The predicted siRNAs hybridized *in silico* with the viral mRNA forming double-chain structures, which are necessary to activate the silencing system; therefore, these siRNAs will be synthesized and their inhibitory effect will be tested against the four DENV serotypes.

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